decreased by 50 per cent at 15 hr, by 44 per cent at 48; histidine decreased 30 per cent at 15 hr, 36 per cent at 48; lysine decreased 48 per cent at 15 hr, 57 per cent at 48; serine decreased 28 per cent at 15 hr, 34 per cent at 48 hr.

It appears, therefore, that alterations in free amino acid levels noted in the later stages of influenza virus infection are not observed during the first 8 hr. These changes which occurred only after extensive viral multiplication may have been the result of diversion of cellular metabolism to viral synthesis. Inflammation of tissue does not appear to be a probable explanation, since inflammatory changes are not ordinarily observed (Burnet, F. M., Australian J. Exptl. Biol. Med. Sci., **19,** 291, 1941). It is possible, however, that the biochemical changes were early manifestations of cellular damage which may be followed by morphological alterations in the cell. It is of interest that only slight alterations in amino acid levels were seen in our experiments (J. Biol. Chem., **211,** 757, 1954) with mumps virus in which virus multiplication appears to occur in the absence of cellular damage.

The authors express their appreciation to Miss Mary Jane Firszt for her assistance in these experiments.

CONJUGAL PAIRING IN *ESCHERICHIA COLI*[1]

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Received for publication December 5, 1955

Previous attempts to correlate genetic with cytological evidence of bacterial sexuality have been indecisive (Lederberg and Tatum, Science, **118,** 169, 1953). Further progress has been achieved with a pair of *Escherichia coli* cultures which are highly interfertile and morphologically distinguishable. They are a motile K-12 Hfr (Cavalli *et al.*, J. Gen. Microbiol., **8,** 89, 1953) and an F- mating type of another strain whose cells are plumper and nonmotile. They are also marked by mutations for Lac, Mal, S, Xyl, Mtl, Ara, V_1 and Gal_2. Both are lambda-sensitive.

Sixteen hour cultures were mixed in the ratio one Hfr to ten F-, diluted in 10 volumes of penassay broth, and incubated 1 hour at 37 C. Reservoir drops of the mixed culture were then prepared as prescribed by de Fonbrune (*Technique de micromanipulation*, Masson, Paris, 1949); however, micropipettes were prepared by hand. The cultures were studied under darkfield at 150 × and under dark phase contrast at higher powers. When the reservoirs were joined to drops of fresh broth, a few per cent of the Hfr cells would carry F- mates, oriented as in figure 1, as they swam away. Other motile cells were stuck in large clumps.

The pairs were isolated to individual droplets. After about an hour, they would disjoin spontaneously, sometimes dividing meanwhile. The

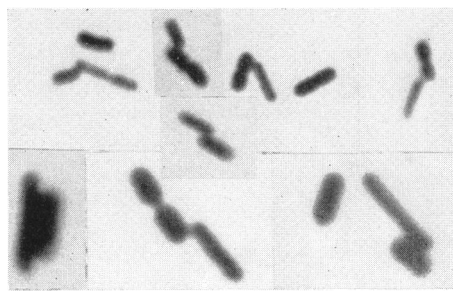

Figure 1. Conjugal pairs, from smear stained with crystal violet. Figures 1, 2, and 3 are at various magnifications; the transverse diameter of the bacteria is about 1 μ.

exconjugant cells were then reisolated and allowed to form larger clones; sometimes, successive daughters were separated first. The following day, the clones were transferred for further propagation and scoring of genetic markers.

Altogether, 279 pairs were isolated. Of these, 222 gave viable progeny from the Hfr exconjugant, none of which showed recombinations; 190

[1] Paper No. 601 of the Department of Genetics. This work has been supported by grants (C2157) from the National Cancer Institute, Public Health Service and from the Research Committee, Graduate School, University of Wisconsin with funds allocated by the Wisconsin Alumni Research Foundation.

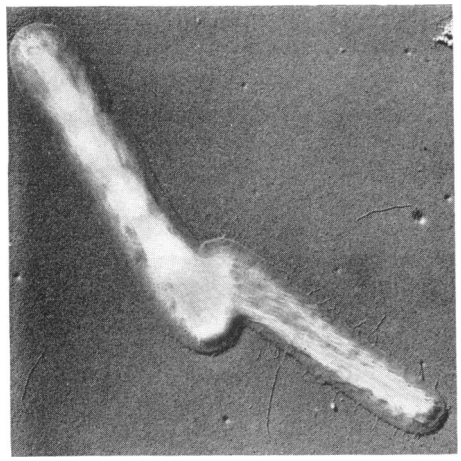

Figure 2. A conjugal pair? Electronmicrograph, chromium-shadowed, of formalin-fixed cells. The assistance of Dr. Paul Kaesberg and Dr. L. L. Cavalli in preparing this photograph is gratefully acknowledged.

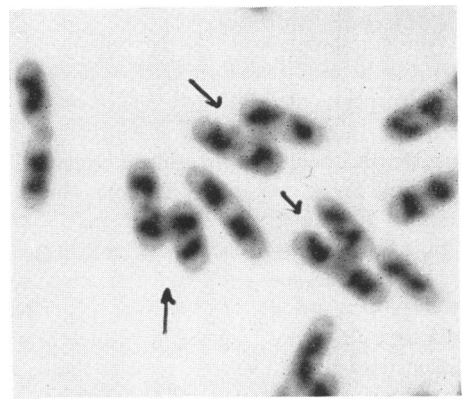

Figure 3. Conjugal pairs? Giemsa stain after acid hydrolysis and osmic fixation. The F- culture in this mating is also from strain K-12.

of the pairs gave viable clones from the F- exconjugant, of which 66 included recombinants as well as the original F- parental combination; 51 of these 66 pairs also engendered viable (and unaltered) clones from the Hfr exconjugant. Control platings of the whole mating population showed that fewer than 1 per cent of the total F- cells were Lac+ S^r recombinants (Lac+ from Hfr; S^r from F-). The distribution of recombinant types was strongly biased in favor of the F- parent. While 63 of the clones included Lac+ S^r, among other recombinants, only one (still Lac- S^r) carried the Gal_2 and Hfr markers of the Hfr parent. As discussed elsewhere (Science, **122,** 920, 1955), haploid segregation data are ambiguous as to the mechanism of the bias. Further details are being collected and analyzed for fuller presentation.

The distinctive motility of the joined pairs cannot be displayed in a still photograph, and figure 1 is intended only to illustrate typical orientations. Owing to difficulties of resolution, the mode of union has not been visualized in living cells. On the other hand, figures 2 and 3 from fixed preparations merely invite apt questions, still being studied, as to the control of preparative artefacts. The principal conclusion that can be justified at present is that recombination is correlated with cell-to-cell pairing, that both exconjugants usually remain viable, and that the recombinants segregate only from the F- parent. These findings are consistent with the postulated transfer of a gametic nuclear element from one multinucleate parental cell to the other.

PRESERVATION OF *DIPLOCOCCUS PNEUMONIAE*

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Received for publication December 9, 1955

A strain of *Diplococcus pneumoniae* Type I has been used in our laboratory for teaching purposes for several years. However, the ability of this organism to produce capsules and its virulence for mice decrease rapidly when a virulent culture is serially transferred in blood broth medium or when lyophilized. It has been found necessary to pass an inoculum obtained from blood broth or lyophilized cultures through mice three to five, or even more, times in order to restore a relatively high degree of virulence.

An attempt has been made to preserve the